13 as well as components of the innate stress response, including the chi-lectins Chi3L1 and Chi3L2 and complement component C3. The major cytokines present were IL-6, GRO, Rantes and MCP-1. Neither IL-1 nor TNF-α was detectable. Several stress response proteins, such as the complement components C1r, C1s and factor H, clusterin, nucleobindin, the chi-lectin Chi3L1 and EDA-domain containing fibronectin were detectable both in control media and following activation of TLR2. Peptidoglycan, TNF-α and IL-1 induced secretion of Chi3L2, the second member of the human chi-lectin family. Chi3L2 was not detectable in control media. Both Chi3L1 and Chi3L2 were present in cartilage from asymptomatic individuals. In contrast to Chi3L1, Chi3L2 levels correlated with the presence of macroscopic signs of damage (Fig. 1).

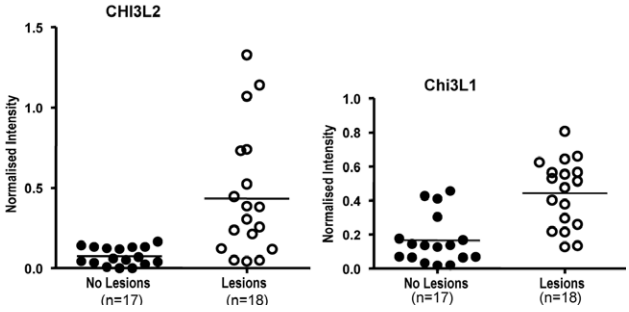

Figure 1. Expression of Chi3L1 and Chi3L2 in articular cartilage from femoral heads of patients undergoing joint replacement. Protein levels were determined by western blotting using specific antisera. The intensity of bands was determined densitometrically and normalized to a Chi3L1 or Chi3L2 standard included in each blot. Cartilage specimens are grouped into those showing macroscopic lesions and lesion-free specimens. The lines represent the mean for each data set.

Conclusions: The increased production of stress response proteins associated with tissue repair and wound healing by chondrocytes suggests that they attempt a repair response similar to that described in other connective tissues. The induction of the chi-lectin Chi3L2 is part of this stress response. Its presence in articular cartilage with early signs of degeneration suggests that Chi3L2 is produced in response to changes in the matrix environment and thus could be a useful indicator for the diagnosis and progression of cartilage erosion.

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ROLE OF THE TRANSCRIPTION FACTOR PITX1, ITS REGULATION AND TARGETS IN OSTEOARTHRITIS

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Purpose: Our previous study demonstrated that partial inactivation of Pitx1 gene led to a progressive formation of OA-like lesions in aging Pitx1+/- mice. To investigate whether Pitx1 plays a role in human osteoarthritis pathogenesis, we analyzed the expression level of Pitx1 in knee cartilage of OA patients and age- and gender-matched control subjects. We observed that Pitx1 mRNA and protein levels were significantly down-regulated in OA patients. The goals of this study are: 1) to elucidate the mechanisms responsible for the down-regulation of Pitx1 in osteoarthritis and 2) to identify genes affected by the loss of Pitx1.

Methods: To look for possible mutations, genomic DNA from OA patients and normal subjects were isolated and 10 kb fragment upstream of Pitx1 initiation transcription site was sequenced. A region in Pitx1 promoter, where a SNP was found in the core of an E2F-response element, was used as bait for DNA pull-down assay followed by mass spectrometry analysis. The proteins which were identified by this method were challenged in different assays either to confirm their presence in the protein-DNA complex (by EMSA, ChIP and co-immunoprecipitation), to analyze their expression level (by RT-PCR and immunohistochemistry) or to determine their ability to affect the regulation of Pitx1 (by transfection assays with luciferase). Finally, to identify potential transcriptional targets of Pitx1, expression level of candidate genes were analysed by RT-PCR using either mouse model or mRNA isolated from normal and OA chondrocytes.
Results: No mutation was found in human Pitx1 promoter to be significantly associated with osteoarthritis. Nevertheless, a SNP, which was found in an E2F-response element caught our attention since E2F1 was known to up-regulate Pitx1. The mass spectrometry analysis allowed us to identify a repressor complex constituted of PHB1, PHB2 and BCoR, which are also known to act as co-repressors. The presence of BCoR tethered to this E2F response element was demonstrated by EMSA and ChIP assays. Since BCoR has never been shown to form a complex with PHB1, we confirmed this result by co-immunoprecipitation using nuclear extracts from chondrocytes of OA patients. By transfection assays using luciferase reporter gene, we were able to show that the complex formed of PHB1, PHB2 and BCoR is functionally capable of down-regulating the expression of Pitx1. Using mRNA isolated from Pitx1+/+, Pitx1-/- mice and OA patients, we were able to show that knockdown of Pitx1 expression resulted in up-regulation of PPAR-gamma, Parp1, Extl3 and Reg1B.
Conclusions: We discovered a new complex formed of PHB1, PHB2 and BCoR which are tethered to an E2F-response element and able to down-regulate the expression of Pitx1 in chondrocytes of OA patients. In pre-OA chondrocytes, the nuclear accumulation of PHB1 could be a primary mechanism leading to OA onset. The subsequent loss of Pitx1 expression could contribute to the progression of OA via the up-regulation of PPAR-gamma, Parp1, Extl3 and Reg1B.

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PHARMACOLOGICAL INVESTIGATION OF THE ROLE OF AGGRECANASES IN HUMAN CARTILAGE DEGRADATION

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Purpose: ADAMTS4 and ADAMTS5 are accepted as the main aggrecanases involved in OA cartilage degradation but the relative contribution of each enzyme in human disease is still under debate. Mouse knockout studies have confirmed the importance of ADAMTS5 in cartilage degradation while ADAMTS4 is generally associated to OA due to its strong upregulation by IL1β. SiRNA experiments indicate that both aggrecanases participate in cartilage degradation in human explants. The present study seeks to further elucidate the role of both aggrecanases in cartilage degradation using selective pharmacological inhibitors.
Methods: Induction of ADAMTS4 and ADAMTS5 expression by different cytokines was studied in cartilage explants using RT-QPCR. Cartilage degradation was induced in human explants by OSM+TNFα or OSM+ IL1β and the role of individual aggrecanases was evaluated by pharmacological inhibition using compounds which were synthesised and selected for high selectivity for ADAMTS4 or ADAMTS5.

Aggrecanase activity was assessed by measuring in the glycosaminoglycan (GAG) release and ARGSVIL neoepitope formation. Explants were further examined by histology after safranin O proteoglycans (PG) staining/light green counterstaining, and by immunohistochemistry for aggrecan NITEGE neoepitope. PG and neoepitope content was then quantified applying a scoring system to individual images.
In addition, cytokine-induced matrix degradation was assessed in a new rat chondrosarcoma cell model ('RCS assay') in which the amount of sulphated proteoglycan was quantified using Alcian blue staining and image processing.
Results: TNFα+OSM or IL1β+OSM strongly induced ADAMTS4 and ADAMTS5 in human cartilage explants. OSM is strictly needed for induction of aggrecanases but not for MMP13.
A specific ADAMTS5 inhibitor (cpd A, IC50 80% inhibition of GAG release, >80% inhibition of neoepitope release at 3μM). In contrast, a selective ADAMTS4 inhibitor (cpd B, IC50 <100 nM) did not inhibited cartilage degradation by more than 50%. Cpd C, a non-selective 3-OH-3-methylpipecolic hydroxamate type metalloproteinase inhibitor completely inhibited GAG and Neoepitope release.
Histological evaluation confirmed that cpd A prevented PG loss and inhibited neoepitope formation in cartilage matrix but not in the pericellular matrix (PM), while cpd B only inhibited neoepitope formation in PM. Cpd C prevented PG loss and inhibited neoepitope formation in both cartilage matrix and PM.
In addition, the compound ranking obtained in the explant model was reproduced with the simple 'RCS assay'.
Conclusions: This study represents the first pharmacological evidence for the importance of ADAMTS5 over ADAMTS4 in human cartilage degradation. Strong induction of ADAMTS5 by cytokines and significant prevention of cartilage degradation by its pharmacological inhibition using a selective inhibitor are demonstrated. This observation underscores the important role of ADAMTS5 in the degradation of human cartilage. Furthermore, these ADAMTS5 effects were recapitulated in a new RCS-based assay, which provides a good substitute for the complex cartilage explant model.
Finally, histological analysis delivered the unexpected observation that matrix aggrecan is subject to different proteolytic events depending on its localization, providing mechanistic information on the cartilage degradation process.

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THE MELANOCORTIN SYSTEM IN HUMAN CHONDROCYTES

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Purpose: Melanocortins (MCs) are structurally related peptides that elicit their actions via binding to melanocortin receptors (MCRs). Originally defined as neurohormones these peptides exhibit an astonishing spectrum of effects on many peripheral cell types and tissues. However, our present knowledge on the function of the MC system in cartilage is scarce. We could show that MC-1R is expressed in human articular cartilage and chondrocytes. α-Melanocyte-stimulating hormone (α-MSH), a prototype of MCs, is capable of regulating a number of important genes involved in extracellular matrix (ECM) composition and inflammation in chondrocytes. The major goal of this study is to define the role of MCs and the MC-1R in the context of osteoarthritis (OA).
Methods: Cartilage slices were aseptically dissected from femoral condyles of OA patients who underwent total knee arthroplasty. Articular chondrocytes isolated from OA-cartilage were proliferated in monolayer until passage one and either cultured in 3D-micromass pellets for 7 days or kept in monolayer. After stimulation for 48h with 10^{-6} M α-MSH only (micromass pellet culture) or in presence of 1 ng/ml IL-1β (monolayer culture); the cDNA copy number of Sox9, collagens, MMPs; TGF-β1,TNF-α and interleukins was assessed with quantitative RT-PCR. POMC, converting enzymes and MC-receptor gene expression was determined by end point RT-PCR and, MC-R1 protein expression *in situ* on cryosections of OA-cartilage by immunohistochemistry. Signal transduction of α-MSH was evaluated by cAMP ELISA and Ca^{2+}-assays using Fura2-AM. Protein concentration of MMP-2, MMP-13, IL-6, TNF-α, TGF-β1 and collagen I and II in cell culture supernatants and cell lysates was determined with ELISA.
Results: Articular OA-chondrocytes express receptors MC-1R, MC-2R and MC-5R plus POMC and converting enzymes PC1, PACE4 and furin convertase. MC-1R protein was preferentially found on chondrocytes from the middle and deep zones of articular cartilage. The MC-1R detected in human chondrocytes appears to be functional as stimulation with 10^{-6} M α-MSH resulted in a moderate but significant increase in intracellular cAMP levels but not in changes of the intracellular Ca^{2+} level. *In vitro* application of 10^{-6} M α-MSH to articular chondrocytes, kept in micromass pellet culture induced gene expression of COL1A1, COL2A1 and COL10A1. Of note, gene expression of MMP-2, MMP-7, MMP-9 and MMP-13 was induced, however, MMP-13 protein concentrations in culture supernatant revealed profound interpatient fluctuations. MMP-2 protein concentration was consistent within the sample collective and remained unaltered in the presence of α-MSH. IL-1β and TNF-α gene expression was reduced, IL-6 remained mostly unchanged and Sox9 and TGF-β1 were induced upon stimulation with α-MSH. IL-6 and TNF-α protein concentration were upregulated. α-MSH attenuates IL-1β-induced expression of IL-4 and IL 8, MMP-2, -9 and -13 in articular OA-chondrocytes.
Conclusions: Human articular chondrocytes are target cells for α-MSH. The effects of α-MSH on cytokine- and MMP expression suggest a chondroprotective role of this neuropeptide in inflammatory and degenerative processes in cartilage. It is conceivable that inflammatory reactions can be mitigated by induction of endogenous melanocortins or administration of α-MSH to the affected joints. The induction pattern of regulatory and structural ECM components as collagens, Sox9, anabolic and catabolic cytokines points towards a function of α-MSH as a trophic factor in skeletal development during endochondral ossification or as anti-inflammatory agent in degenerative joint pathologies.

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THE FUNCTION OF p53 ON HUMAN CHONDROCYTE APOPTOSIS IN RESPONSE TO SHEAR STRESS

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Purpose: Chondrocyte apoptosis plays an important role in cartilage degeneration in osteoarthritis (OA), and mechanical injury to cartilage induces chondrocyte apoptosis. p53 has been identified as a 53 kDa cellular protein and 'ultimate tumor suppressor gene'. p53 is recognized as a pivotal regulatory protein that responds to a variety of signals and recruits an array of biochemical activities to trigger diverse biological responses, most notably cell cycle arrest and apoptosis. In response to DNA damage, p53 expression is upregulated, increases transcriptional activity, and initiates apoptosis signals. p53-regulated apoptosis-inducing protein 1 (p53AIP1) is one of the p53-regulated genes, and is activated in response to severe DNA damage with the phosphorylation of p53 at Ser-46. In this study, we speculated that p53 and p53AIP1 might play an important role in DNA damage-induced apoptosis of articular carti-